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Non-human mtDNA helps to exculpate a suspect in a homicide case

Christa Nussbaumer *, Irina Korschineck

Ingenetix Ltd., Vienna, Austria

Abstract. In January 2005 the dead body of a young female was found close to a highway in the south of Austria. Since the corpse had been set on fire using an accelerant, the victim has not been identified so far. Part of the sweater the woman was wearing was left and showed few short animal hairs. The investigations led the police to a young man who was already on remand due to a property offense. On the basis of morphological comparison, the hairs found on the sweater of the victim were suspected to derive either from an animal out of the group of minks and martens (*Mustelidae*) or from a dog (*Canidae*). Since hardly any of the hairs showed a root, mtDNA analysis had to be performed on the few hairs collected from the victim's sweater and on the hairs found in the suspect's car. The questions posed by the court were as follows: which species do the hairs on the sweater originate from, and secondly: do the hairs on the sweater and the hairs in the car belong to the same individual.

Keywords: mtDNA; Cytochrome *b*; Control region; *Canis familiaris*; Animal hair; Cycle sequencing

1. Introduction

This case report shows the results of a mtDNA analysis requested by the court investigating the homicide of a young unidentified woman. Cycle sequencing of a part of the cytochrome *b* gene located on the mtDNA was performed in order to determine the animal species of the hairs found on the victim's corpse [1]. Furthermore a part of the hypervariable control region was analyzed from all the evidence samples originating from dogs [2]. The haplotypes were aligned with the haplotype of reference hairs from dogs found in the suspect's car.

* Corresponding author. Tel.: +43 699 196 85 858.
E-mail address: Christa.Nussbaumer@ingenetix.com (C. Nussbaumer).

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2. Material and methods

2.1. DNA extraction

DNA extraction of the hairs was done according to the instruction manual of the QiaAmp DNA mini kit (Qiagen, Germany). DTT was added during lysis at 56 °C.

2.2. DNA amplification

2.2.1. Cytochrome b

5 μl of the DNA extracts were amplified using 0.5 μM universal primers (forward_14841: 5′ CCA TCC AAC ATC TCA GCA TGA TGA AA 3′ and reverse_15149: 5′ GCC CCT CAG AAT GAT ATT TGT CCT CA 3′, ~300 bp) in 25 μl reaction volume at 55 °C annealing temperature for 37 cycles.

2.2.2. Control region

5 μl of DNA extracts were amplified using 0.5 μM dog specific primers (forward_15926: 5′ TCA AAG CTT ACA CCA GTC TTG TAA ACC 3′ and reverse_16498: 5′CCT GAA GTA GGA ACC AGA TG 3′, ~570 bp) in 25 μl reaction volume at 55 °C annealing temperature for 37 cycles.

2.2.3. DNA sequencing

5 μl of purified PCR products were sequenced from both ends with BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) according to the manufacturer's protocol. Sequence analysis of the fluorescently labeled fragments was performed on an ABI 310 capillary electrophoresis instrument. The sequences were aligned and assembled using SeqScape v2.5 (Applied Biosystems).

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Canis fam.      TACTAGGAGTATGCTTGATTCTACAGATTCTAACAGGTTTATTCTTAGCTATGCACTATA
victim_Canis    TACTAGGAGTATGCTTGATTCTACAGATTCTAACAGGTTTATTCTTAGCTATGCACTATA
car_Canis.      TACTAGGAGTATGCTTGATTCTACAGATTCTAACAGGTTTATTCTTAGCTATGCACTATA
car__Felis.     TTCTAGGAGTTTGCCTAATCTTACAAATCCTCACCGGCCTCTTTTTGGCCATACACTACA
                * ******** *** * **  **** ** ** ** **  * ** ** ** ** ***** *

Canis fam.      CATCGGACACAGCCACAGCTTTTTCATCAGTCACCCACATCTGCCGAGACGTTAACTACG
victim_Canis    CATCGGACACAGCCACAGCTTTTTCATCAGTCACCCACATCTGCCGAGACGTTAACTACG
car_Canis.      CATCGGACACAGCCACAGCTTTTTCATCAGTCACCCACATCTGCCGAGACGTTAACTACG
car_Felis.      CATCAGACACAATAACCGCCTTTTCATCAGTTACCCACATCTGTCGCGACGTTAATTATG
                **** ******   ** ** *********** *********** ** ******** ** *

Canis fam.      GCTGAATTATCCGCTATATGCACGCAAATGGCGCTTCCATATTCTTTATCTGCCTATTCC
victim_Canis    GCTGAATTATCCGCTATATGCACGCAAATGGCGCTTCCATATTCTTTATCTGCCTATTCC
car_Canis.      GCTGAATTATCCGCTATATGCACGCAAATGGCGCTTCCATATTCTTTATCTGCCTATTCC
car_Felis.      GCTGAATCATCCGATATTTACACGCCAACGGAGCTTCCATATTCTTTATCTGCCTGTACA
                ******* ***** *** * ***** ** ** ********************** * *

Canis fam.      TACATGTAGGACGAGGCCTATATTACGGATCCTATGTATTCATAGAAACATGAAACATTG
victim_Canis    TACATGTAGGACGAGGCCTATATTACGGATCCTATGTATTCATAGAAACATGAAACATTG
car_Canis.      TACATGTAGGACGAGGCCTATATTACGGATCCTATGTATTCATAGAAACATGAAACATTG
car_Felis.      TACATGTAGGACGGGGAATGTACTACGGCTCCTACACCTTCTCAGAGACATGAAACATTG
                ************** **  * ** ***** *****    ***  *** **************
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Fig. 1. Alignment (CLUSTALX v 1.83) of the partial mtDNA sequences of cytochrome *b* from hairs found on the victim, hairs from the car and the *Canis familiaris* sequence from the NCBI genbank (accession number NC_002008).

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base       11111111111111111111111111111111111111111111111111111111
position   55555555555555555555555555555555555555555555555555555555
           44444444445555555555555555556666666666666666666666666666
           36678899990112222222233445589111122222333333334444455 5
           64553401368382345678904143765125801578012456890358901 2
            .                     .
            1                     1

reference  A-TTCAACGCCGATTCTCCCCT-CCATACTTCATCTATCACGATTTTAAAATCG
genbank

victim     ..................................C.................
car_1      ..........................................A........
car_2      .C.............T...........C.........T....G.G.....A
r.hair_dog ..........................................A........
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Fig. 2. Different haplotypes of partial sequences of the control region from hairs found on the victim and hairs from the car referenced to the *Canis familiaris* sequence from the NCBI genbank (NC_002008—only the polymorohic sites are shown). The reference sequence of the hair from the dog of the car owner (r.hair_dog) is also displayed. Each haplotype was derived from at least 2–6 different hairs sequenced from both ends.

3. Results and discussion

3.1. Species determination by sequencing of cytochrome b

The universal primers which amplify the target gene of a broad range of vertebrates were successfully applied to the evidence samples. The nucleotide sequence was subjected to the BLAST search and could be classified as originating from a dog. One of the hairs found in the car of the suspect matched with a cat (see Fig. 1).

3.2. mtDNA haplotype analysis of the control region

Several dog hairs from the victim's sweater and several dog hairs from the suspect's car were successfully sequenced within the control region. All fragments from the victim's sweater showed a single haplotype whereas the hairs from the suspect's car showed two different haplotypes (see Fig. 2). Neither of these sequences matched the haplotype of the hairs from the victim's body. One of the haplotypes found in the car matched the reference hair from the dog of the car owner. The haplotype of the hair from the victim's body and the two haplotypes from the hairs in the car differed from each other by at least two mutations.

References

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